

Disruption of Fibroblast Growth Factor Receptor (FGFR) Signaling as an Approach to Prostate Cancer Gene Therapy

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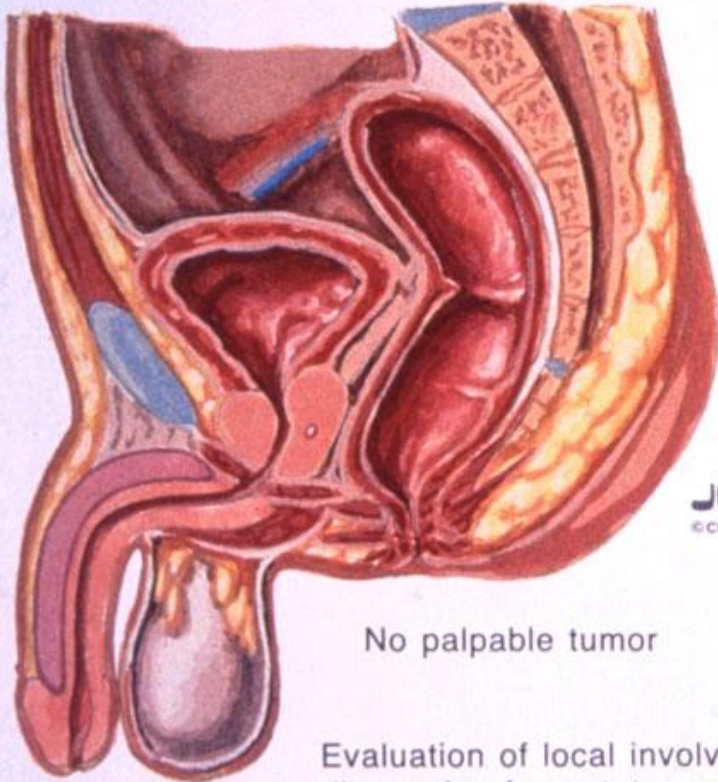
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Clinical Staging of Prostate Cancer

Primary tumor



No palpable tumor

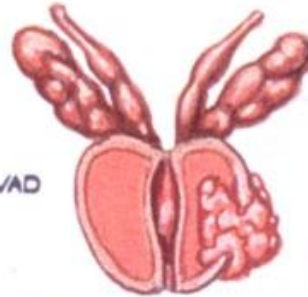
Evaluation of local involvement and spread beyond confines of gland follows diagnosis of prostate cancer



Confined to gland



Capsular invasion



Extension beyond
confines of gland



Invasion of neighbor-
ing structures

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- **Fibroblast growth factor (FGF) family:**
 - includes at least 22 different genes (FGF1-22) encoding related polypeptide mitogens.
 - can stimulate the proliferation of a wide variety of cells.
 - are expressed abundantly in prostate tissue.
 - are required for prostate cells to grow *in vitro*.

- **FGF Receptors:**

- located in cell membrane
- bind FGFs and mediate the action of FGFs.
- include four high affinity tyrosine kinase receptors (FGFR1-4).
- involved in the regulation of cell growth, development, and differentiation in a variety of tissues.
- have increased tyrosine kinase activity in response to binding FGF
- work in pairs at cell surface

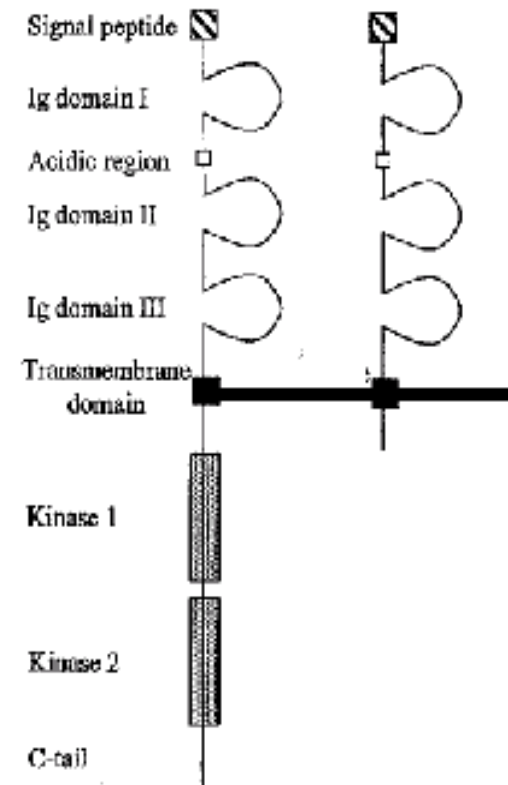
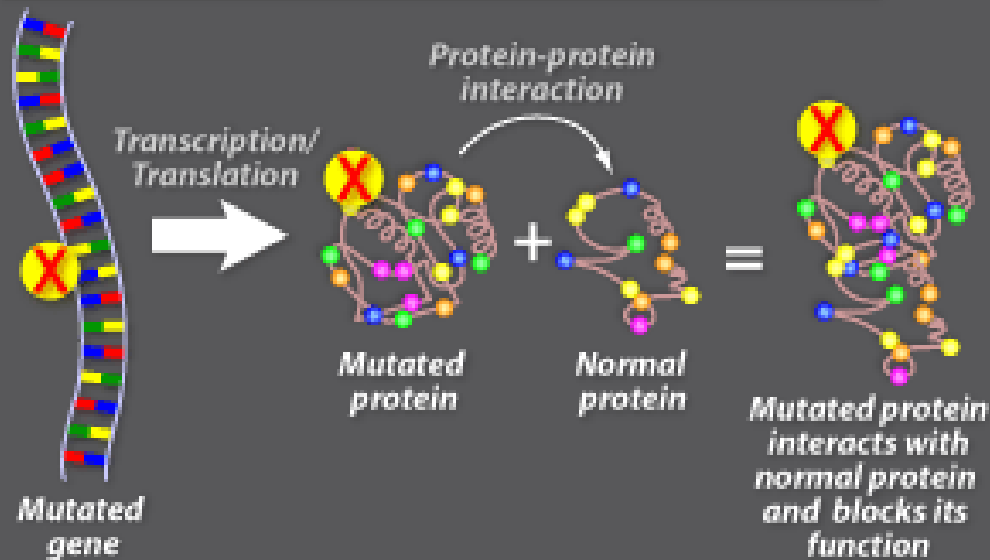
HYPOTHESIS

- Prostate cancer cells are dependent upon FGFR signaling for survival and proliferation.
- Disruption of this signaling pathway by expression of a DN FGFR protein might contribute to death of cancer cells and can be used as gene therapy adjuvant to current treatment options.
- Analysis of gene expression profile in DN FGFR transfected cells might help our understanding of how DN FGFR works and the differentially expressed genes determined by microarray analysis can be used as targets for prostate cancer therapy.

Dominant negative FGF receptor

What is a Dominant Negative Mutation?

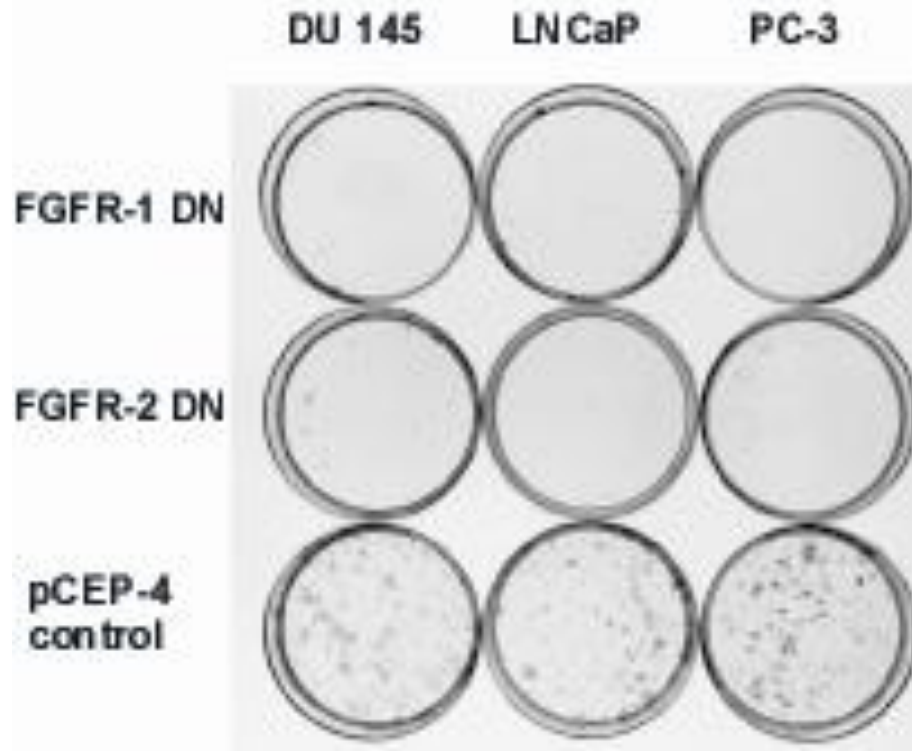
Answer: A mutated gene produces a mutated protein that blocks the function of a normal protein.

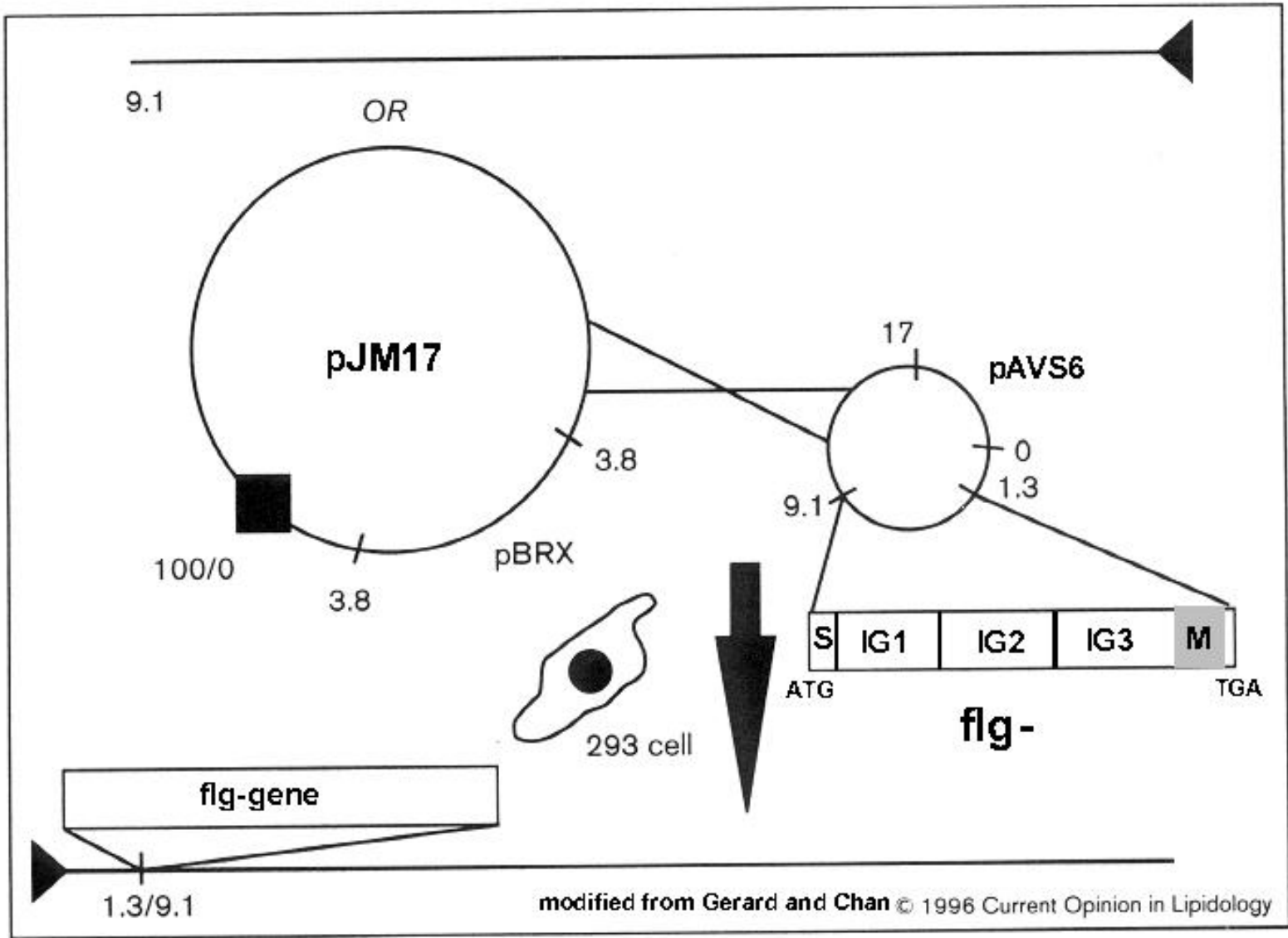




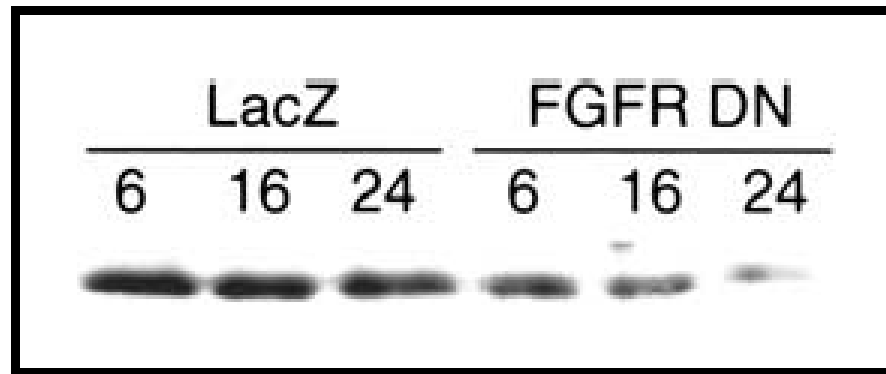
YEDİTEPE ÜNİVERSİTESİ
HASTANESİ

COLONY FORMATION



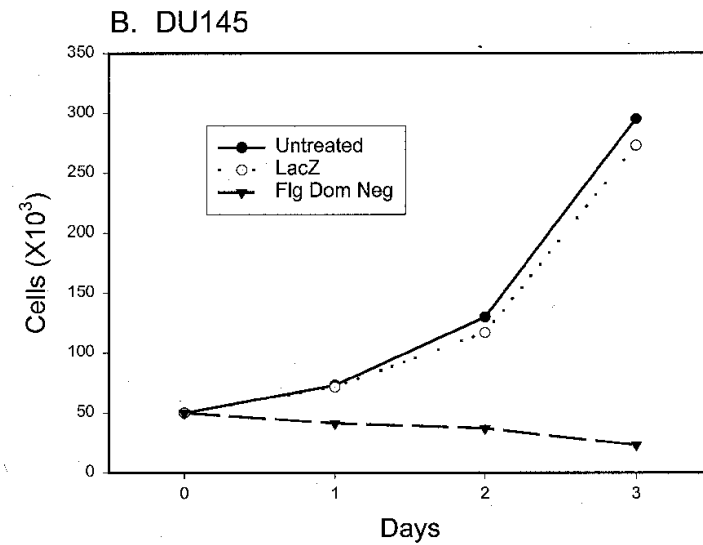
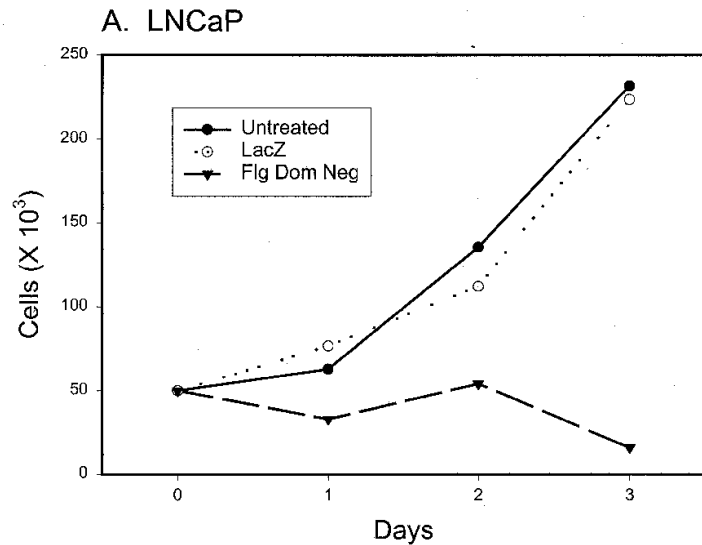


FGFR DN markedly decreases phosphorylated FGFR-1



IP with α -PT, probed with α -FGFR1

FGFR DN inhibits proliferation of prostate cancer cells



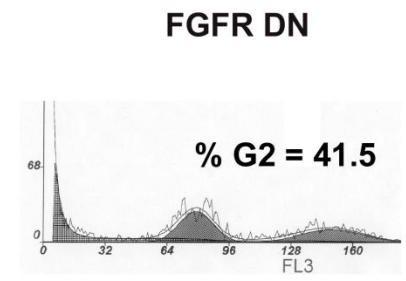
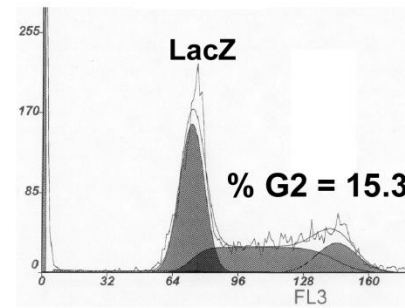
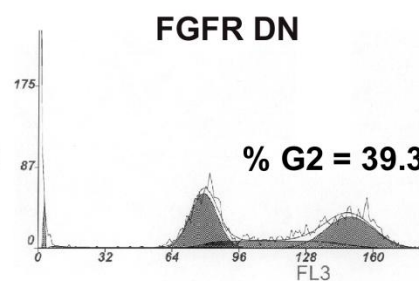
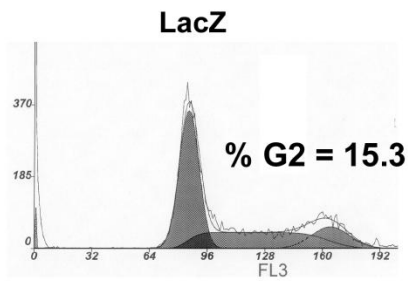


FGFR DN blocks cells at G2/M

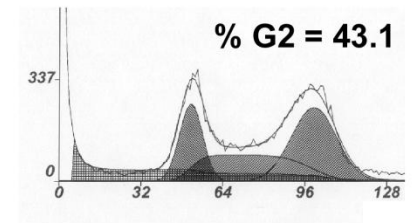
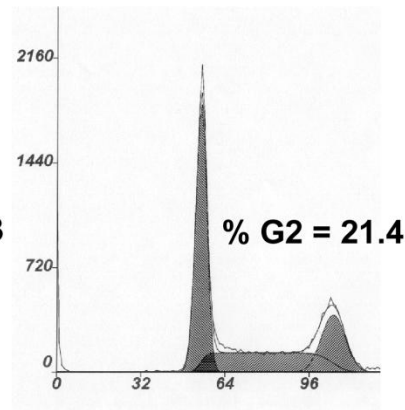
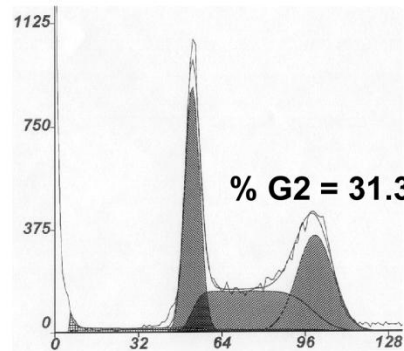
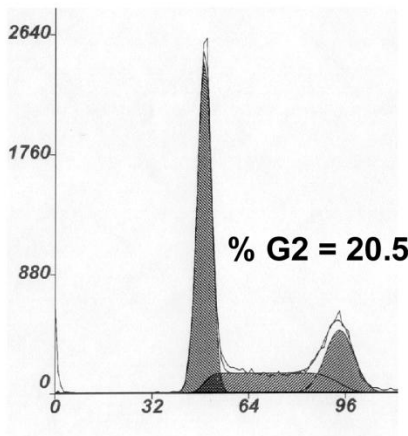
A: LNCaP

24 hrs

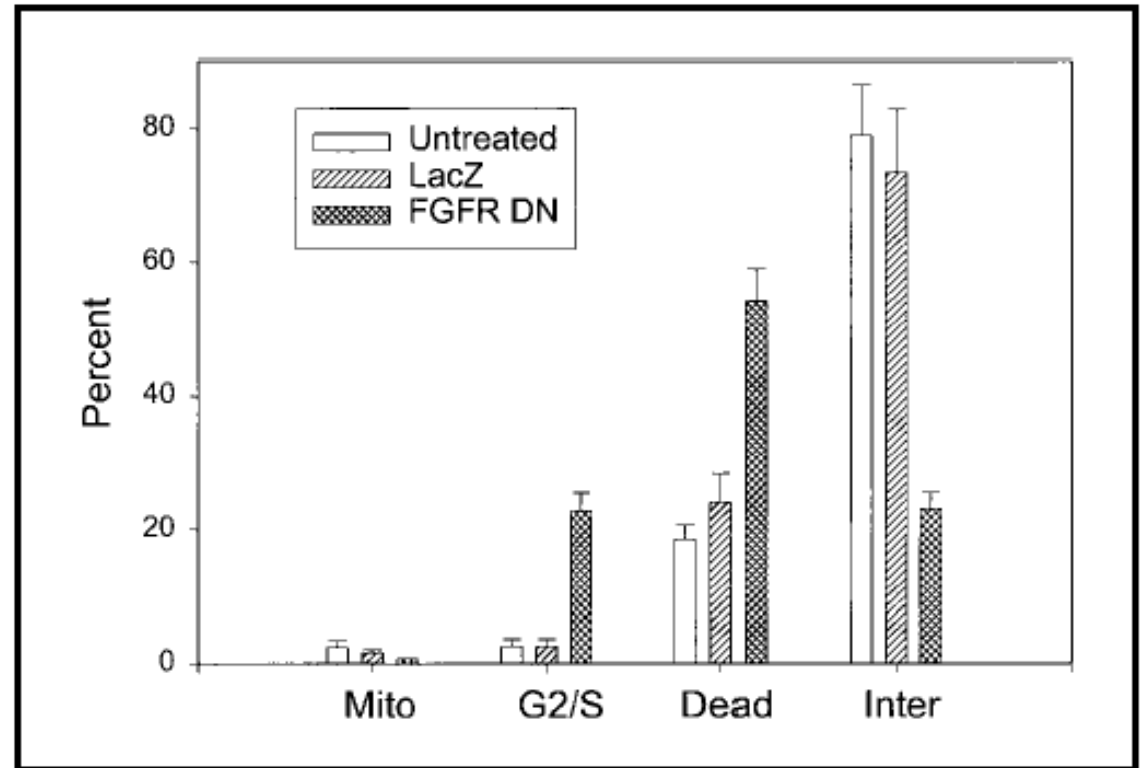
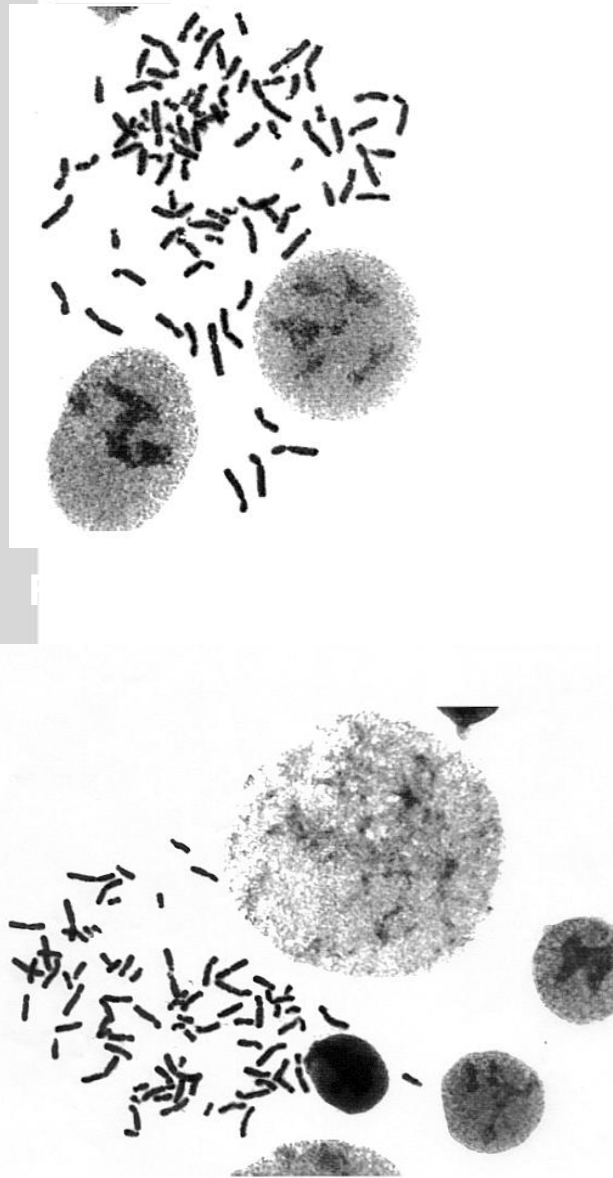
48 hrs



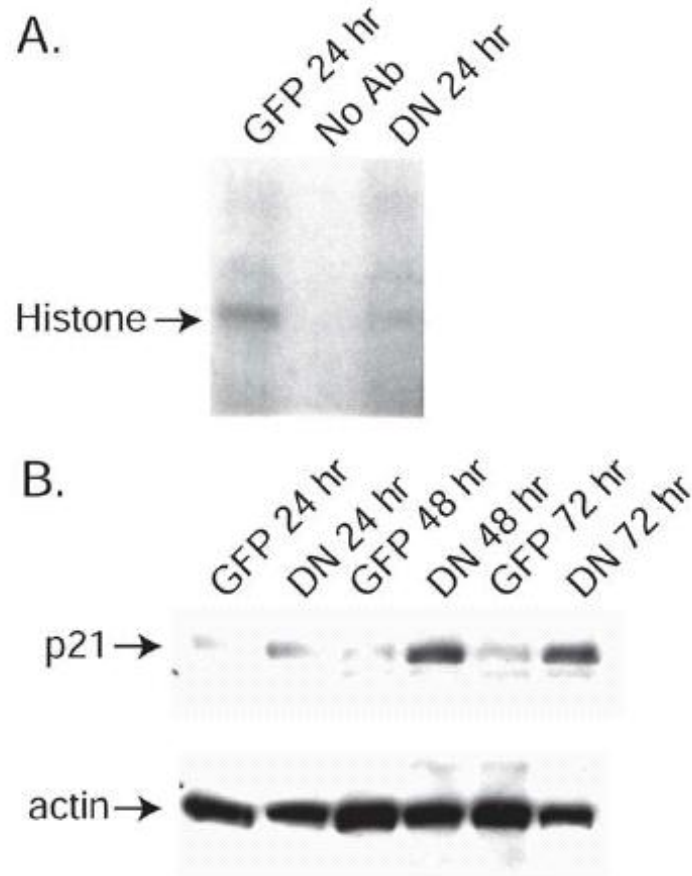
B: DU145

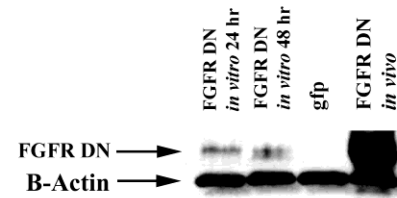
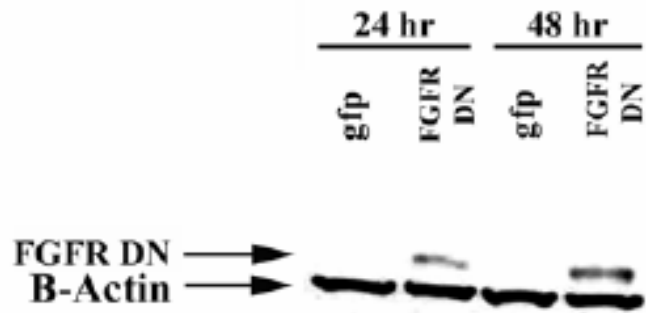


FGFR DN treated cells showed more cells at G2



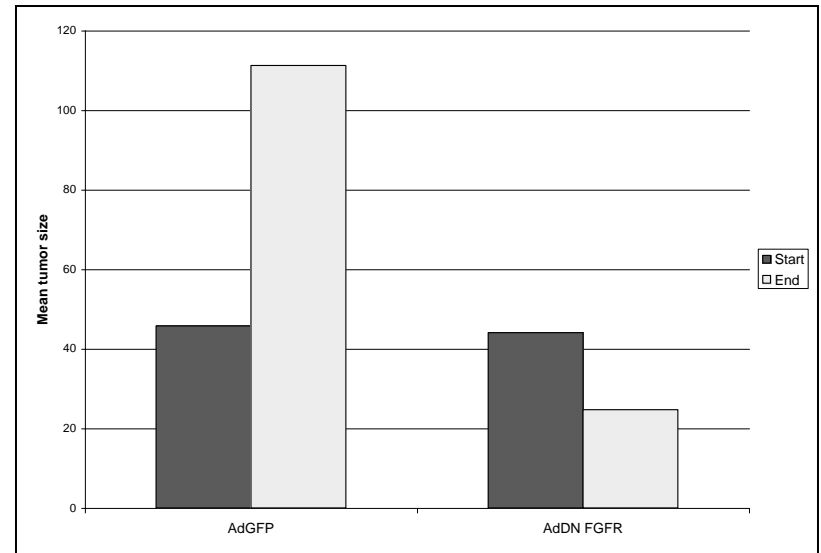
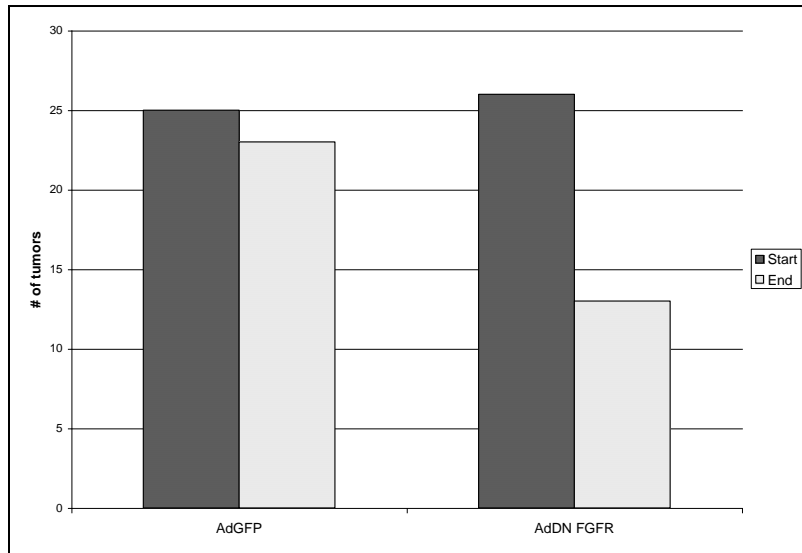
FGFR DN decrease cdc2 kinase activity and increase p21 expression







Treatment of prostate cancer xenografts with AdDN FGFR

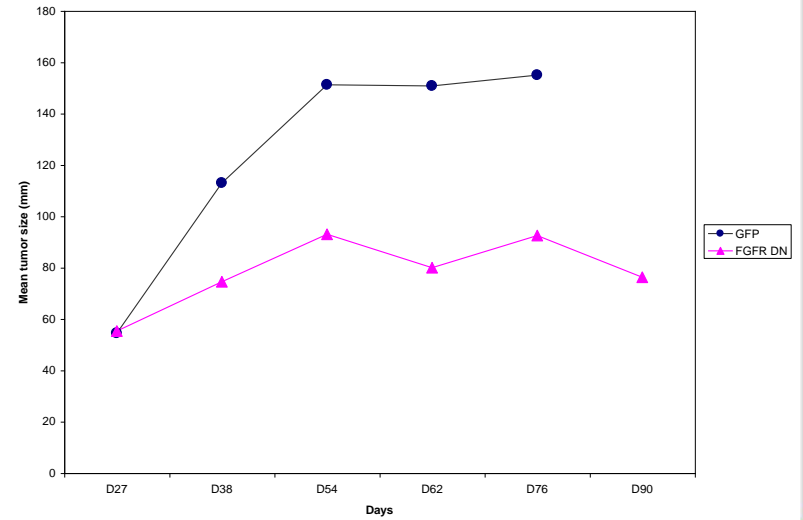
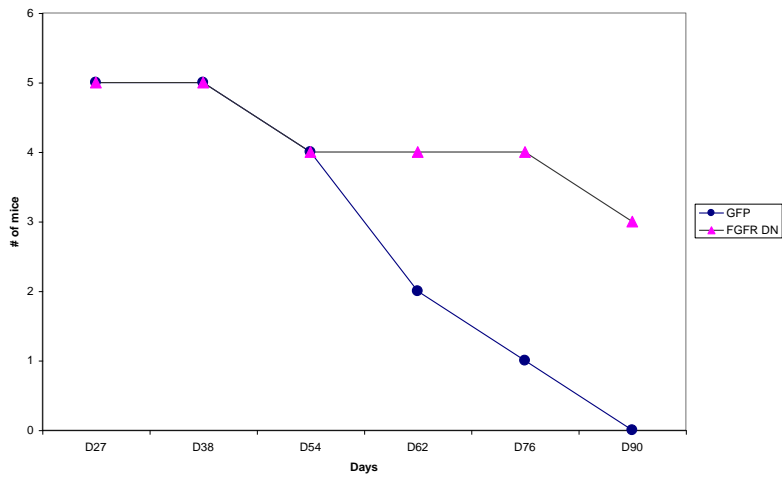


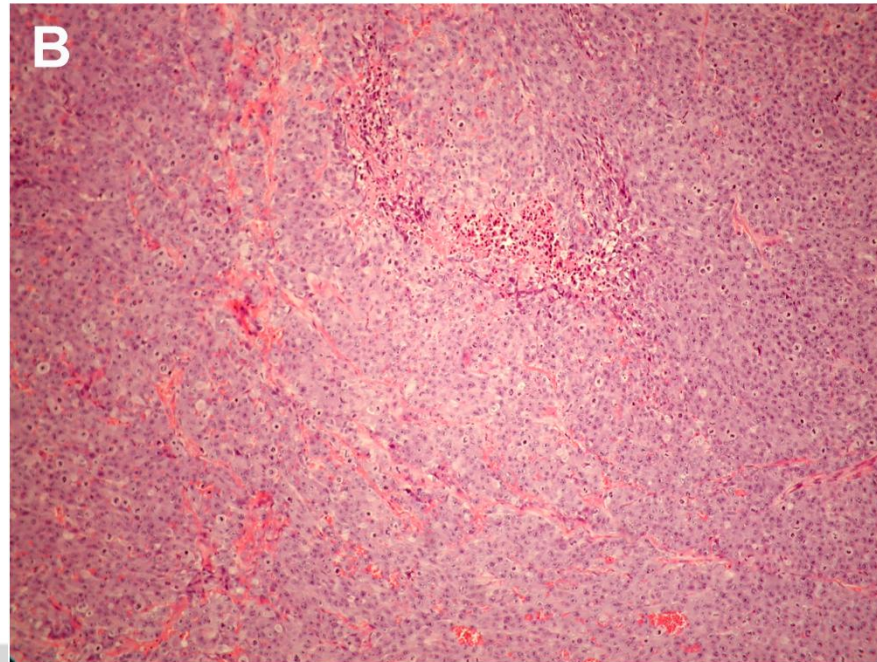
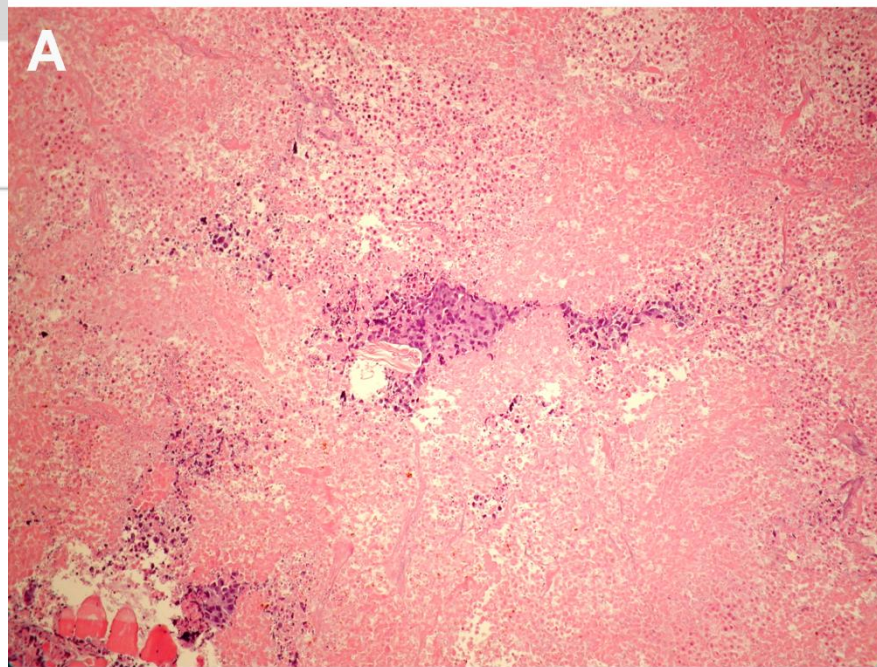
AdDN FGFR

AdGFP Control



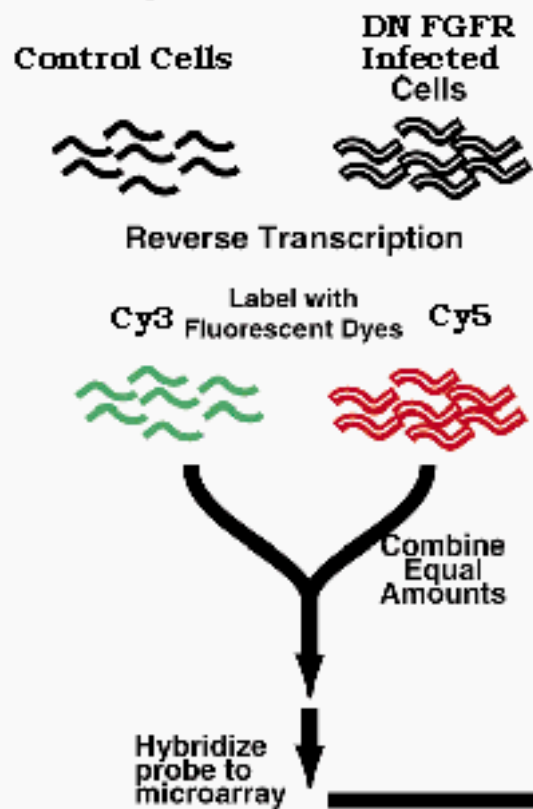
Effect of AdFGFR DN on LNCap cells in vivo



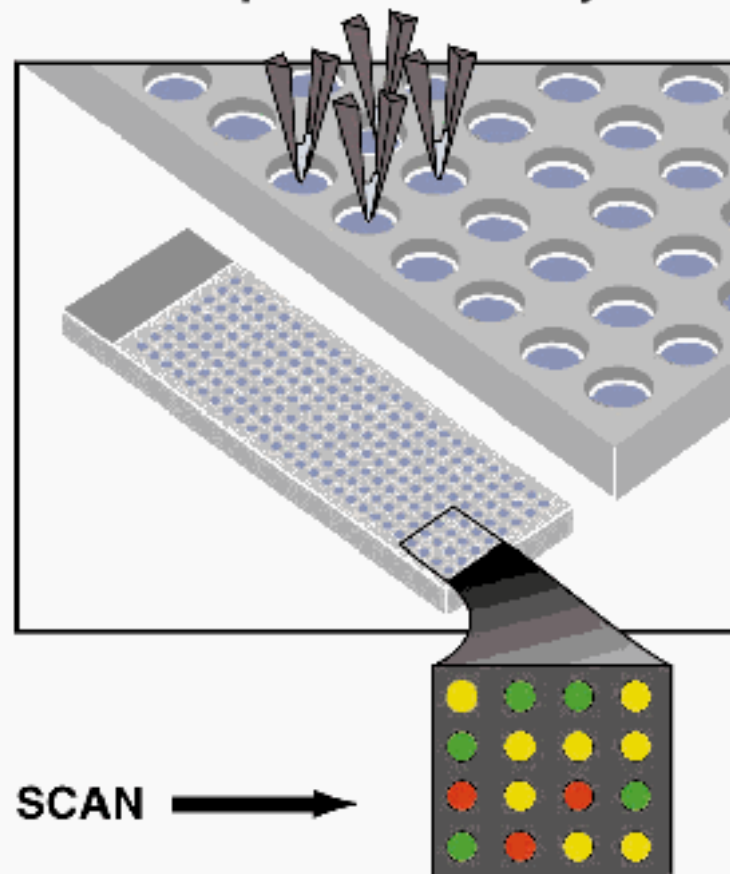


Micro-array Experiment

Prepare cDNA Probe



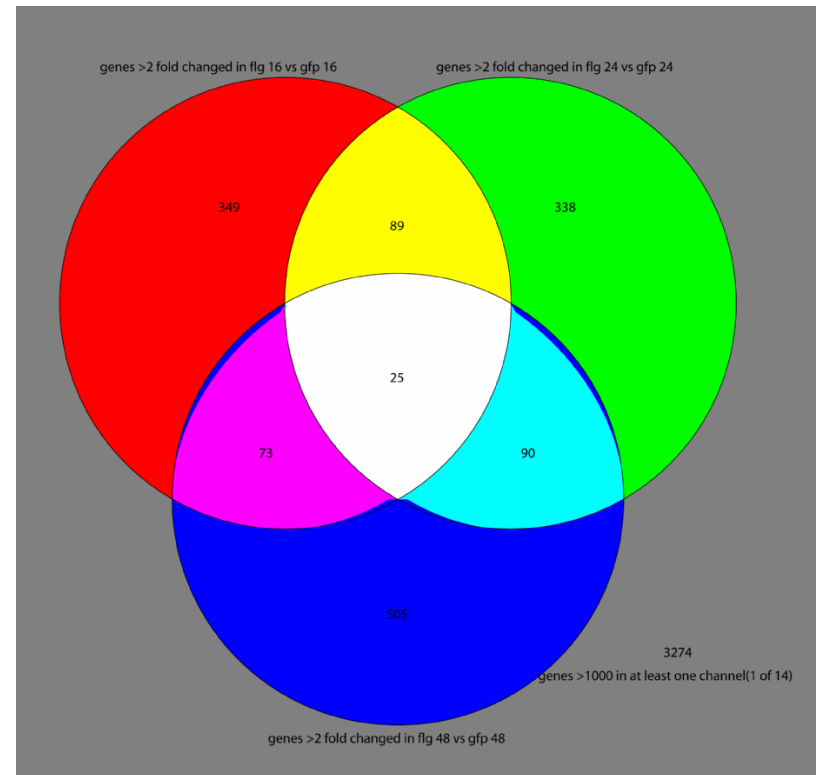
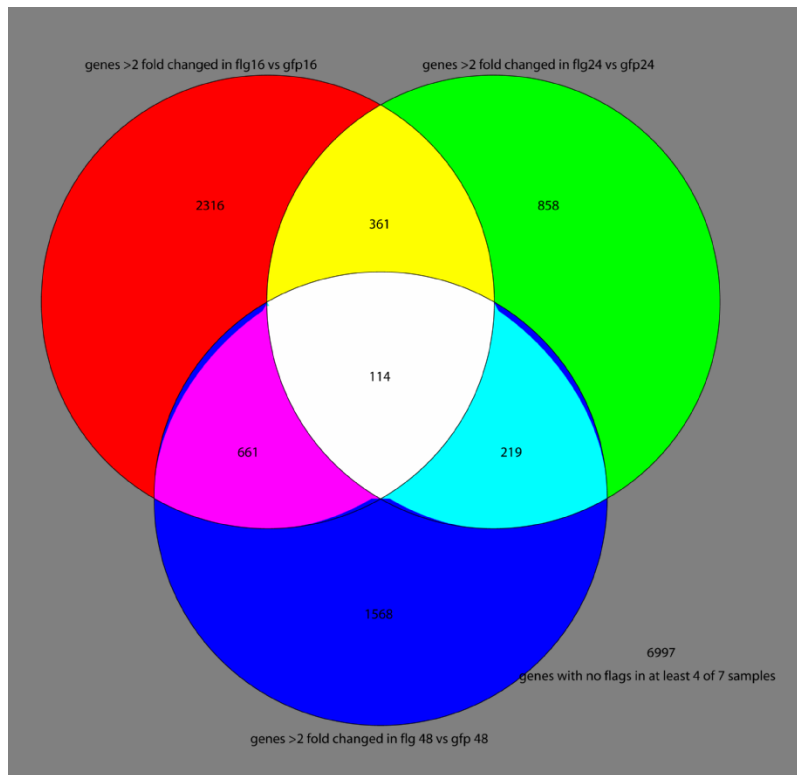
Prepare Microarray



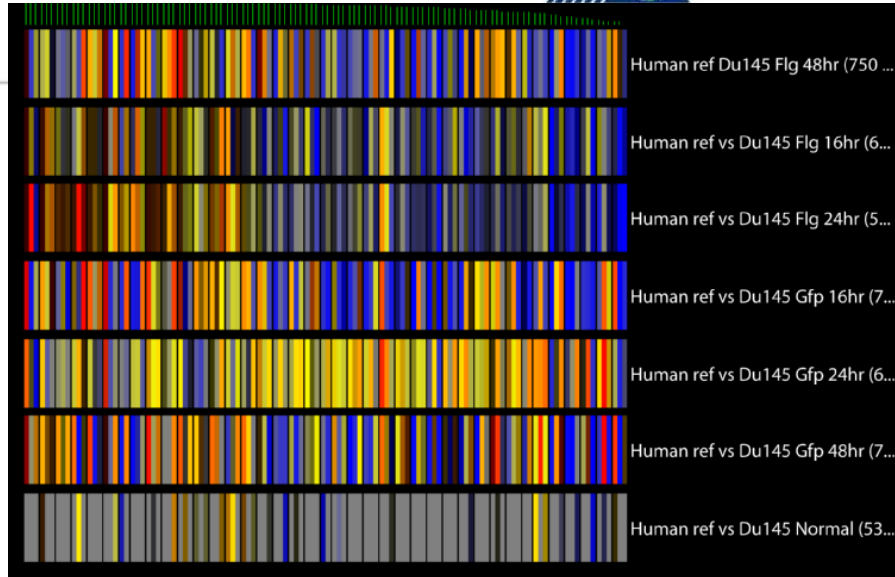


DU145

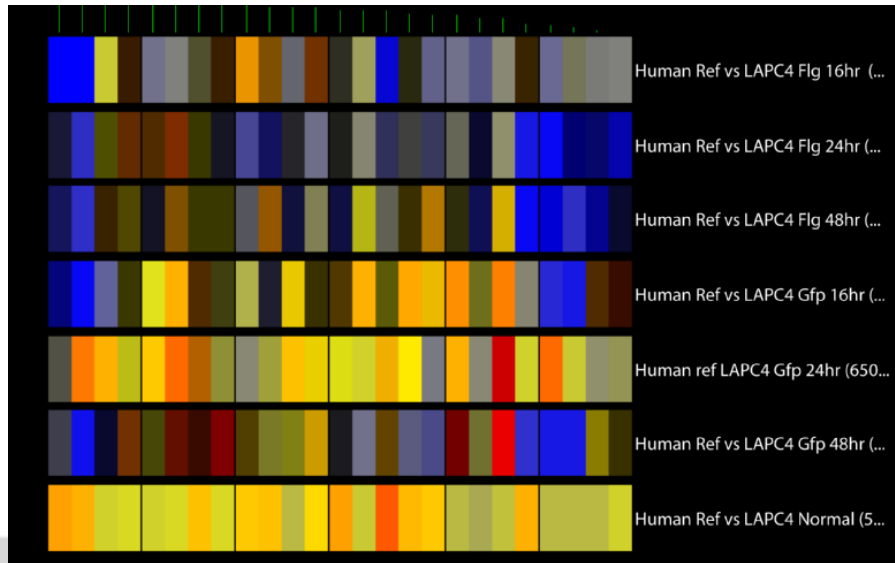
LAPC4



DU145

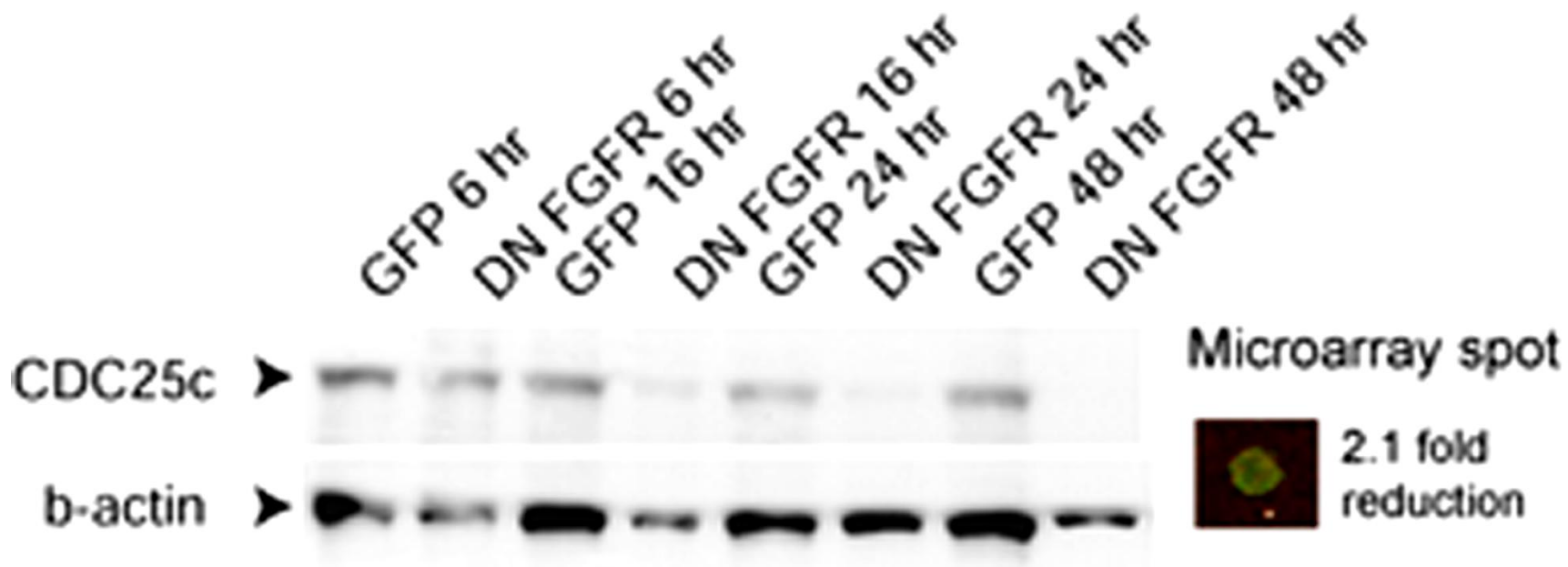


LAPC4

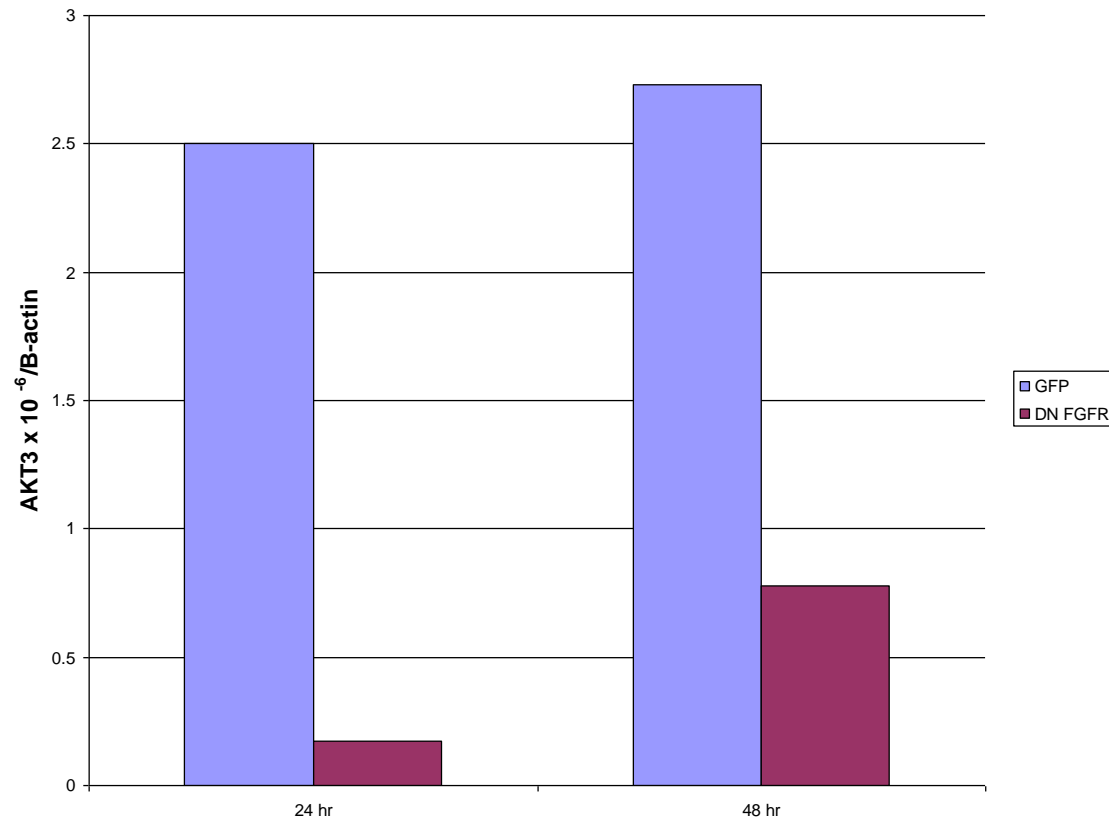


Hs.65029	0.522	growth-arrest-specific protein
Hs.248	0.518	cot proto-oncogene
Hs.153752	0.516	CDC25B; CDC25HU2; M-phase induc
Hs.155324	0.507	matrix metalloproteinase 11 (MM
Hs.251674	0.499	rho6 protein
Hs.77432	0.498	epidermal growth factor recepto
Hs.30942	0.498	ephrin-B2 precursor; EPH-relate
Hs.106283	0.496	insulin-like growth factor bind
Hs.41688	0.496	dual-specificity protein phosph
Hs.59106	0.494	p53-dependent cell growth regul
Hs.77597	0.494	serine/threonine-protein kinase
Hs.656	0.491	CDC25C; M-phase inducer phosph
Hs.75379	0.491	high-affinity glutamate transpo
Hs.8724	0.491	NDR protein kinase
Hs.73986	0.489	CDC-like kinase 2 (CLK2)
Hs.101448	0.481	metastasis-associated protein 1
Hs.251674	0.479	cyclin-dependent kinase 5 activ
Hs.166681	0.465	ets-related protein tel; ets tr
Hs.79347	0.463	epidermal growth factor recepto
Hs.79126	0.463	guanine nucleotide-binding prot
Hs.173664	0.458	ERBB2 receptor protein-tyrosine
Hs.107169	0.453	insulin-like growth factor bind
Hs.79078	0.451	mitotic feedback control protei
Hs.81248	0.436	Wilms' tumor protein (WT33; WT1
Hs.75862	0.427	mothers against dpp homolog 4 (
Hs.75770	0.424	retinoblastoma-associated prote
Hs.103042	0.422	microtubule-associated protein
Hs.180911	0.416	Homo sapiens ribosomal protein





FGFR DN down-regulates AKT3



CONCLUSIONS

- **FGFs are important survival factors for prostate cancer cells.**
- **AdDN FGFR inhibits growth of human prostate cancer cell lines LNCaP and DU-145 *in vitro* and LNCaP *in vivo* as tested.**
- **This inhibition is due to the cell cycle arrest at G2 check point. Degradation of cdc25C and up-regulation of p21 molecules in DN FGFR infected cells might contribute to this inhibition.**

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